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Glucose Intolerance, as Reflected by Hemoglobin A_{1c} Level, Is Associated With the Incidence and Severity of Transplant Coronary Artery Disease

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OBJECTIVES The possible effect of plasma hemoglobin A_{1c} (HbA_{1c}) on the development of transplant coronary artery disease (TxCAD) was investigated.

BACKGROUND Glucose intolerance is implicated as a risk factor for TxCAD. However, a relationship between HbA_{1c} and TxCAD has not been demonstrated.

METHODS Plasma HbA_{1c} was measured in 151 adult patients undergoing routine annual coronary angiography at a mean period of 4.1 years after heart transplantation. Intracoronary ultrasound (ICUS) was also performed in 42 patients. Transplant CAD was graded by angiography as none, mild (stenosis in any vessel ≤30%), moderate (31% to 69%), or severe (≥70%) and was defined by ICUS as a mean intimal thickness (MIT) ≥0.3 mm in any coronary artery segment. The association between TxCAD and established risk factors was examined.

RESULTS Plasma HbA_{1c} increased with the angiographic grade of TxCAD (5.6%, 5.8%, 6.4%, and 6.2% for none, mild, moderate, and severe disease, respectively; $p < 0.05$ for none vs. moderate or severe) and correlated with disease severity ($r = 0.24$, $p < 0.05$). The HbA_{1c} level was higher in patients with MIT ≥0.3 mm than in those with MIT <0.3 mm (6.4% vs. 5.7%, $p < 0.05$). Multivariate logistic regression analysis identified HbA_{1c} as an independent predictor of TxCAD, as detected by angiography or ICUS (odds ratios 1.9 and 2.4, 95% confidence intervals 1.5 to 6.3 [$p = 0.010$] and 1.3 to 4.2 [$p < 0.005$], respectively).

CONCLUSIONS Persistent glucose intolerance, as reflected by plasma HbA_{1c}, is associated with the occurrence of TxCAD and may play an important role in its pathogenesis. (J Am Coll Cardiol 2004; 43:1034–41) © 2004 by the American College of Cardiology Foundation

Transplant coronary artery disease (TxCAD) is the leading cause of mortality in long-term survivors of heart transplantation. Evidence suggests that risk factors for TxCAD include hyperlipidemia (1,2), body mass index (BMI) (3,4), and donor age (5), with the rejection incidence (6,7) being only weakly correlated with TxCAD. Glucose intolerance and associated hyperlipidemia occur frequently as a consequence of the action of immunosuppressive drugs used to treat heart transplant patients (8). Studies of diabetes mellitus (DM) in animal models have suggested that hyperglycemia may be an important factor in the pathogenesis of TxCAD (1). However, little is known about the relationship between hyperglycemia and the incidence and severity of TxCAD in humans. Hemoglobin A_{1c} (HbA_{1c}) is a useful index of glucose intolerance and hyperglycemia, even when fasting glucose concentrations are normal (9,10). We have now examined the hypothesis that the severity of glucose intolerance, as reflected by HbA_{1c} concentration, correlates with the severity of TxCAD.

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METHODS

Study subjects. The study population comprised 151 consecutive individuals (88 men and 63 women) who underwent heart transplantation at ages 16 to 61 years between January 1990 and February 1995 at Stanford University School of Medicine. To reduce possible bias due to the duration of post-transplant survival, we included only patients who were between 3.5 and 4.7 years (mean 4.1) post-transplantation. All subjects underwent coronary angiography and measurement of plasma HbA_{1c} and serum lipid concentrations. Intracoronary ultrasound (ICUS) was performed in a subgroup of 42 patients (27 men and 15 women) aged 26 to 60 years. The demographics of the study subjects, including pretransplant heart disease, donor age, immunosuppressive treatment, and DM treated before or after transplantation, were recorded. Treated DM was defined as any history of DM that required pharmacologic intervention with insulin or oral antidiabetic agents. Immunosuppression in all patients included prophylactic OKT3 during the initial seven days after transplantation and maintenance thereafter on a standard three-drug regimen of cyclosporine, prednisone, and azathioprine. In patients who experienced recurrent rejection episodes, azathioprine was replaced by mycophenolate mofetil, and, in some instances,

Abbreviations and Acronyms

BMI = body mass index
CMV = cytomegalovirus
DM = diabetes mellitus
HbA_{1c} = hemoglobin A_{1c}
HDL = high-density lipoprotein
ICUS = intracoronary ultrasound
ISHLT = International Society for Heart and Lung Transplantation
LDL = low-density lipoprotein
MIT = mean intimal thickness
TxCAD = transplant coronary artery disease

cyclosporine was replaced by tacrolimus. All patients received antiplatelet therapy (aspirin 80 or 325 mg/day); 91 patients received hydroxymethylglutaryl coenzyme A reductase inhibitors; and 48 patients received diltiazem (120 mg/day). The study was approved by the Institutional Review Board of Stanford University School of Medicine, and written, informed consent was obtained from all participants.

Angiographic and ICUS classification. Routine surveillance coronary angiograms obtained at a mean of 4.1 years after transplantation were reviewed by two angiographers independently, and a consensus on the grading of TxCAD was reached, according to the following classification: none, mild (≤30% stenosis in any vessel), moderate (31% to 69% stenosis), or severe (≥70% stenosis or severe peripheral pruning). Coronary artery intimal thickness was measured for the region between the left main coronary artery and the mid portion of the left anterior descending coronary artery by ICUS at the time of angiography in a subset of 42 patients. The mean intimal thickness (MIT) was calculated from the difference between the lumen area and total vessel area. On the basis of the Stanford Classification (11), TxCAD was defined as MIT ≥0.3 mm in any segment.

Evaluation of potential risk factors for TxCAD. Plasma HbA_{1c} and glucose concentrations and serum concentrations of triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol were measured after a 12-h fast on the day of coronary angiography. Donor age at the time of transplantation, increase in recipient BMI after transplantation, and rejection incidence were recorded. The change (increase) in BMI after transplantation (ΔBMI) was defined as BMI at the time of angiography minus BMI at the time of transplantation. An average rejection score for each patient was derived from International Society for Heart and Lung Transplantation (ISHLT) grading (0 = 0, 1A = 1, 1B = 2, 2 = 3, 3A = 4, 3B = 5, 4 = 6) and was calculated as the sum of the rejection scores for each biopsy divided by the total number of biopsies. The cumulative doses (total drug amount per kilogram of body weight) of cyclosporine, tacrolimus, and prednisone were also determined.

Statistical analysis. Data are expressed as the mean value ± SD. The distributions of key variables, normal or otherwise, were evaluated by normal distribution plots and histograms to determine whether parametric or nonparametric tests should be used for analysis of group differences. One-way analysis of variance was used to assess overall differences, and the Bonferroni correction for multiple comparisons was applied to examine differences among the four groups stratified by angiographically determined TxCAD severity. Differences between two groups were assessed by the unpaired *t* test or chi-squared test. The Spearman correlation coefficient was determined to evaluate whether TxCAD severity, as estimated from angiograms, was correlated with the plasma concentration of HbA_{1c}.

Univariate logistic regression analysis was used to select the potential independent predictive factors for TxCAD (defined as ≥70% stenosis by angiography or MIT ≥0.3 mm by ICUS) for inclusion in multivariate analysis. The covariables examined included gender and the occurrence of DM treated before or after transplantation as categorical variables, and donor age, recipient age, baseline BMI, ΔBMI, average rejection score, and fasting levels of HbA_{1c}, glucose, triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol as continuous variables. Univariate predictors of TxCAD, as determined by angiography or ICUS, with a p value of <0.05, were entered into a multivariate logistic regression model with stepwise selection. Differences were considered statistically significant at $p < 0.05$.

To assess the correlation between TxCAD identified by angiography and that identified by ICUS, we analyzed the relationship between stenosis determined by angiography and MIT determined by ICUS with the use of Pearson's correlation coefficient. In addition, the correlation between the TxCAD classifications derived from angiography and those from ICUS was evaluated with the Spearman rank correlation coefficient. The MIT cutoff value of ≥0.3 mm, determined by ICUS, was used to differentiate between angiographically determined none or mild (≤30% stenosis) TxCAD and moderate or severe (≥31% stenosis) TxCAD, as well as between none/mild/moderate (≤69% stenosis) and severe (≥70% stenosis) TxCAD. The sensitivity (true positive/[true positive + false negative]), specificity (true negative/[true negative + false positive]), and predictive accuracy ([true positive + true negative]/total group) were determined and expressed as percentages.

Linear regression analysis was used to determine whether a correlation existed between the plasma HbA_{1c} concentration and cumulative doses of cyclosporine, tacrolimus, or prednisone.

RESULTS

Continuous variables analyzed in the present study were normally distributed. Demographic characteristics of the

Table 1. Characteristics of Study Subjects Stratified by Grade of Transplant Coronary Artery Disease as Determined by Angiography

Characteristic	Grade of TxCAD				p Value
	None (n = 49)	Mild (n = 30)	Moderate (n = 41)	Severe (n = 28)	
Recipient age (yrs)*	41.1 ± 9.4	38.7 ± 7.1	42.3 ± 9.6	43.4 ± 11.2	0.24
Male/female	30/19	16/15	24/19	18/10	0.74
Donor age (yrs)	26.0 ± 10.0	26.5 ± 8.2	27.0 ± 9.8	31.8 ± 12.2	0.09
Baseline BMI (kg/m^2)*	23.7 ± 3.4	24.3 ± 3.4	24.1 ± 3.6	24.5 ± 4.1	0.72
Primary diagnosis (%)					
IDCM	44.9	48.4	46.5	53.6	0.65
IHD	34.7	38.9	34.9	30.7	0.46
Other	20.4	12.7	18.6	15.7	0.25
Drugs (%)					
Cyclosporine	79.6	73.3	79.1	71.4	0.62
Tacrolimus	20.4	25.3	20.9	28.6	0.49
Azathioprine	73.6	74.1	79.1	67.1	0.12
MMF	20.7	22.9	20.9	25.5	0.53
Prednisone	61.2	51.6	46.5	53.6	0.73
HMG-CoA RI	67.3	61.2	51.2	60.7	0.48
Diltiazem	28.6	35.5	30.2	35.7	0.78
Treated DM (%)					
Pretransplant	8.2	9.7	16.3	13.9	0.65
Post-transplant	14.3	19.3	13.9	17.9	0.90

*Values at the time of heart transplantation. Data are presented as the mean value ± SD or percentage of patients in each subgroup.

BMI = body mass index; DM = diabetes mellitus; HMG-CoA RI = hydroxymethylglutary-CoA reductase inhibitors; IDCM = idiopathic cardiomyopathy; IHD = ischemic heart disease; MMF = mycophenolate mofetil; TxCAD = transplant coronary artery disease.

study subjects did not differ significantly among the groups stratified by either the grade of TxCAD determined angiographically (Table 1) or MIT determined by ICUS (Table 2).

Table 2. Characteristics of Study Subjects Stratified by Mean Intimal Thickness, as Determined by Intracoronary Ultrasound

Characteristic	MIT		p Value
	<0.3 mm (n = 20)	≥0.3 mm (n = 22)	
Recipient age (yrs)*	48.8 ± 6.9	54.9 ± 5.3	0.066
Male/female	14/6	13/9	0.31
Donor age (yrs)	30.9 ± 8.9	36.4 ± 9.8	0.065
Baseline BMI (kg/m^2)*	24.2 ± 4.1	23.9 ± 3.5	0.80
Primary diagnosis (%)			
IDCM	50.0	59.1	0.65
IHD	50.0	39.3	0.46
Other	0	1.6	0.025
Drugs (%)			
Cyclosporine	75.0	72.3	0.62
Tacrolimus	25.0	27.3	0.49
Azathioprine	80.0	68.1	0.34
MMF	20.0	31.9	0.40
Prednisone	40.0	50.0	0.73
HMG-CoA RI	65.0	63.6	0.43
Diltiazem	30.0	45.5	0.31
Treated DM (%)			
Pretransplant	10.0	18.2	0.92
Post-transplant	15.0	18.2	0.44

*Values at the time of heart transplantation. Data are presented as the mean value ± SD or percentage of patients in each subgroup.

MIT = mean intimal thickness; other abbreviations as in Table 1.

Comparison of fasting HbA_{1c}, lipid, and glucose concentrations, as well as ΔBMI and drug doses, among patients stratified by TxCAD severity. The fasting concentrations of HbA_{1c}, lipids, and glucose, as well as ΔBMI, for the study subjects are presented according to angiographic (Table 3) or ICUS findings (Table 4). The plasma concentration of HbA_{1c} was significantly higher in patients with moderate or severe TxCAD, as determined by angiography, than in those with no disease. It was also significantly higher in patients with moderate to severe TxCAD than in those with none/mild TxCAD. The severity of TxCAD, as determined by angiography, was correlated with the plasma concentration of HbA_{1c} (Spearman $r = 0.24$, $p = 0.0434$) (Fig. 1). The ΔBMI was significantly greater in patients with moderate or severe TxCAD than in those with no disease or mild TxCAD. Similarly, ΔBMI in patients with moderate to severe TxCAD was greater than that in those with none/mild TxCAD. Both the plasma HbA_{1c} concentration and ΔBMI were significantly greater in subjects with MIT ≥0.3 mm than in those with MIT <0.3 mm. The cumulative doses of drugs known to affect glucose tolerance (cyclosporine, tacrolimus, and prednisone) showed no correlation with the plasma concentration of HbA_{1c} or with TxCAD (data not shown).

Association between established risk factors and TxCAD. Multiple logistic regression analysis was performed for TxCAD, defined as either ≥70% stenosis determined by angiography (Table 5) or MIT ≥0.3 mm determined by ICUS (Table 6), as the dependent variable and for risk

Table 3. Plasma Concentrations of Hemoglobin A_{1c} and Glucose, Serum Lipid Concentrations, and Change in Body Mass Index Stratified by Grade of Transplant Coronary Artery Disease as Determined by Angiography

	Grade of TxCAD				
	None (n = 49)	Mild (n = 31)	Moderate (n = 43)	Severe (n = 28)	
Variable	None/Mild (n = 80)		Moderate/Severe (n = 71)		p Value
HbA_{1c} (%)	5.6 ± 1.0*	5.8 ± 1.0	6.4 ± 1.8	6.2 ± 1.6	0.08
	5.7 ± 1.0†		6.3 ± 1.7		0.012
Glucose (mg/dl)	132.4 ± 70.1	135.7 ± 61.0	129.2 ± 56.7	124.8 ± 30.0	0.90
	133.7 ± 66.3		127.5 ± 47.4		0.52
Triglycerides (mg/dl)	221.8 ± 53.7	231.6 ± 76.1	224.7 ± 65.6	202.5 ± 46.6	0.73
	227.9 ± 68.8		226.3 ± 59.9		0.74
Total cholesterol (mg/dl)	188.5 ± 52.5	203.6 ± 63.6	195.6 ± 66.4	202.5 ± 46.6	0.72
	195.6 ± 66.4		198.3 ± 59.1		0.74
HDL cholesterol (mg/dl)	45.9 ± 12.1	42.0 ± 10.5	43.2 ± 7.6	40.7 ± 6.2	0.14
	44.2 ± 11.5		42.1 ± 7.1		0.21
LDL cholesterol (mg/dl)	128.6 ± 40.0	151.3 ± 40.9	137.8 ± 45.4	135.0 ± 58.7	0.31
	140.0 ± 41.7		136.7 ± 50.8		0.70
ΔBMI (kg/m^2)	0.3 ± 5.0‡	0.4 ± 5.0†	2.1 ± 5.1	3.4 ± 4.6	0.035
	0.4 ± 5.0§		2.6 ± 4.9		0.0059

*$p < 0.05$ versus either moderate or severe. †$p \leq 0.05$ versus moderate/severe. ‡$p < 0.05$ versus either moderate or severe. §$p < 0.01$ versus moderate/severe. All analyte concentrations were determined under fasting conditions. Data are presented as the mean value ± SD.

HbA_{1c} = hemoglobin A_{1c}; HDL and LDL = high- and low-density lipoprotein, respectively; other abbreviations as in Table 1.

factors that showed a significant association with TxCAD identified by angiography or ICUS on univariate analysis. Among the variables included in the multivariate analysis, only the plasma HbA_{1c} concentration was significantly associated with TxCAD identified by angiography. Similarly, only the plasma HbA_{1c} concentration and, to a lesser extent, ΔBMI were significantly associated with TxCAD identified by ICUS on multivariate analysis.

Concordance between TxCAD identified by angiography or ICUS. Pearson's correlation analysis revealed that the percent stenosis determined by coronary angiography was correlated with MIT determined by ICUS ($r = 0.614$, $p < 0.0001$) (Fig. 2A). The angiographic stratification of TxCAD was also correlated with the classification of TxCAD as MIT ≥0.3 mm by ICUS (Spearman $r = 0.74$, $p < 0.0001$) (Fig. 2B). The MIT cutoff value of ≥0.3 mm, as determined by ICUS, was used to differentiate between patients with none/mild TxCAD and those with moderate to severe TxCAD, as determined by angiography, yielding sensitivity, specificity, and predictive accuracy values of 94.4% (17 of 18 subjects), 79.2% (19 of 24 subjects), and 85.7% (36 of 42 subjects), respectively. Similarly, differentiation between patients with none/mild/moderate TxCAD and those with severe TxCAD revealed sensitivity, specificity, and predictive accuracy values of 100% (9 of 9 subjects), 60.6% (20 of 33 subjects), and 69.0% (29 of 42 subjects), respectively.

DISCUSSION

Transplant CAD is the leading cause of death or retransplantation among long-term survivors of heart transplantation. Up to 50% of transplant recipients have angiographically detectable CAD, and even more patients exhibit intimal thickening on examination by ICUS, five years after transplantation (12–14). We have now investigated risk factors for development of TxCAD and demonstrate a correlation between glucose intolerance, as reflected by

Table 4. Plasma Concentrations of Hemoglobin A_{1c} and Glucose, Serum Lipid Concentrations, and Change in Body Mass Index Stratified by Mean Intimal Thickness, as Determined by Intracoronary Utrasound

	MIT		
Variable	<0.3 mm (n = 20)	≥0.3 mm (n = 22)	p Value
HbA_{1c} (%)	5.7 ± 1.1	6.4 ± 2.1	0.041*
Glucose (mg/dl)	122.5 ± 48.5	129.4 ± 49.9	0.68
Triglycerides (mg/dl)	221.8 ± 53.7	223.2 ± 52.5	0.86
Total cholesterol (mg/dl)	183.2 ± 52.5	180.3 ± 57.2	0.86
HDL cholesterol (mg/dl)	50.1 ± 13.2	45.0 ± 4.4	0.11
LDL cholesterol (mg/dl)	138.6 ± 41.9	135.7 ± 69.6	0.88
ΔBMI (kg/m^2)	−0.2 ± 4.5	3.5 ± 4.8	0.015*

*$p < 0.05$. Data are presented as the mean value ± SD.

Abbreviations as in Tables 1 and 3.

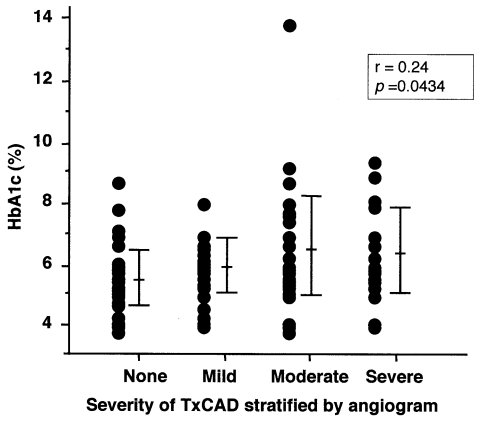

Figure 1. Relationship between the grade of TxCAD stratified by coronary angiography and plasma HbA_{1c} concentration.

increased plasma HbA_{1c} levels, and the occurrence of TxCAD. The results of the present study are consistent with our previous observations linking aspects of insulin resistance syndrome to the development of TxCAD (15). Specifically, we showed that plasma glucose and insulin concentrations measured 2 h after oral challenge with 75 g of glucose were significantly higher in patients with TxCAD than in those without the disease (8). Our present results further suggest that, even in the presence of near-normal fasting glucose levels, chronic mild elevation of plasma glucose in heart transplant patients may contribute to the development of TxCAD.

Although some studies have demonstrated a high prevalence of glucose intolerance among TxCAD patients (8,16), many others have failed to show a correlation between TxCAD and fasting blood glucose concentration. One possible reason for this discrepancy is a lack of consistency in evaluation of glucose intolerance in transplant patients. The sporadic measurements of fasting blood glucose in such individuals in the transplant clinic setting are unreliable for assessing long-term glucose intolerance, because the results may vary markedly depending on both the patient's medical regimen and the time since transplantation. Although testing of oral glucose tolerance is a better approach, it is labor-intensive. However, several studies (9,10) have shown that HbA_{1c} is a reliable index of persistent hyperglycemia.

We have now used HbA_{1c} as a measure of persistent hyperglycemia to investigate whether the severity of glucose intolerance is correlated with the occurrence and severity of TxCAD, as assessed by either angiography or ICUS. In addition to postprandial hyperglycemia, obesity is an important feature of insulin resistance syndrome. In the present study, we found that ΔBMI was greater in patients with TxCAD than in those without it, even though BMI at the time of transplantation did not differ significantly between the two groups. This observation is consistent with the previous demonstration that weight gain after transplantation is an important risk factor for TxCAD (16). An increase in BMI is consistent with the insulin resistance syndrome induced by immunosuppressive drugs in heart transplant patients (15). The importance of obesity in the context of insulin resistance as a risk factor for cardiovascular disease in the general population has also been emphasized (17,18).

We did not detect a significant correlation between TxCAD and any of the lipid abnormalities previously identified as risk factors for this condition. This discrepancy

Table 5. Univariate and Multiple Logistic Regression Analysis of Risk Factors for Transplant Coronary Artery Disease, as Identified by Angiography

Variable	OR (95% CI)	p Value
Univariate logistic regression analysis		
Gender (male)	2.8 (1.4–9.2)	0.73
Pretransplant treated DM	1.1 (0.5–2.9)	0.079
Post-transplant treated DM	1.8 (1.05–3.3)	0.61
Donor age (yrs)	2.3 (1.6–3.3)	0.002
Recipient age (yrs)	1.1 (0.6–2.0)	0.81
Baseline BMI (kg/m^2)	1.2 (1.0–1.3)	0.58
ΔBMI (kg/m^2)	4.1 (1.4–14.8)	0.02
Rejection score	1.7 (0.9–3.4)	0.02
HbA_{1c} (%)	2.08 (1.3–3.3)	0.002
Glucose (mg/dl)	1.2 (0.29–4.9)	0.71
Triglycerides (mg/dl)	1.9 (1.3–2.9)	0.004
Total cholesterol (mg/dl)	1.65 (1.06–2.6)	0.03
HDL cholesterol (mg/dl)	1.4 (0.46–4.4)	0.56
LDL cholesterol (mg/dl)	0.89 (0.7–1.3)	0.9
Multiple logistic regression analysis		
Donor age (yrs)	0.99 (0.94–1.05)	0.73
ΔBMI (kg/m^2)	1.34 (0.92–3.4)	0.059
Rejection score	0.98 (0.95–1.02)	0.98
HbA_{1c} (%)	1.9 (1.5–6.3)	0.010
Triglycerides (mg/dl)	0.99 (0.91–1.25)	0.74
Total cholesterol (mg/dl)	1.00 (0.97–1.03)	0.99

CI = confidence interval; OR = odds ratio; other abbreviations as in Tables 1 and 3.

Table 6. Univariate and Multiple Logistic Regression Analysis of Risk Factors for Transplant Coronary Artery Disease, as Identified by Intracoronary Ultrasound

Variable	OR (95% CI)	p Value
Univariate logistic regression analysis		
Gender (male)	1.75 (0.54–5.6)	0.34
Pretransplant treated DM	1.4 (0.42–5.2)	0.09
Post-transplant treated DM	1.5 (0.47–4.7)	0.42
Donor age (yrs)	4.2 (1.1–16.3)	0.019
Recipient age (yrs)	0.92 (0.29–2.86)	0.88
Baseline BMI (kg/m^2)	1.8 (1.05–3.3)	0.18
ΔBMI (kg/m^2)	2.0 (1.3–3.1)	0.001
Rejection score	0.71 (0.52–4.4)	0.006
HbA_{1c} (%)	2.2 (1.5–3.2)	<0.001
Glucose (mg/dl)	1.6 (1.3–1.8)	0.41
Triglycerides (mg/dl)	1.1 (0.6–2.0)	0.059
Total cholesterol (mg/dl)	1.5 (1.03–2.3)	0.009
HDL cholesterol (mg/dl)	1.6 (0.46–6.4)	0.08
LDL cholesterol (mg/dl)	1.2 (0.26–6.3)	0.28
Multiple logistic regression analysis		
Donor age (yrs)	1.03 (1.00–1.12)	0.73
ΔBMI (kg/m^2)	1.5 (1.0–2.1)	0.041
Rejection score	0.62 (0.25–1.98)	0.98
HbA_{1c} (%)	2.4 (1.3–4.2)	0.0043
Total cholesterol (mg/dl)	0.99 (0.98–1.01)	0.99

Abbreviations as in Tables 1, 3, and 5.

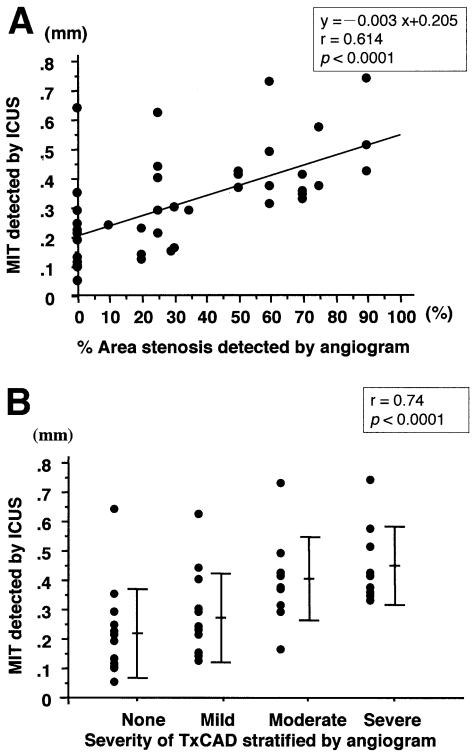

Figure 2. Relationships between percent stenosis determined by coronary angiography and mean intimal thickness (MIT) determined by intracoronary ultrasound (ICUS) **(A)** and between the severity of transplant coronary artery disease (TxCAD) stratified by coronary angiography and MIT determined by ICUS **(B)**.

might be due to the fact that statin therapy was routinely initiated early after heart transplantation in all of the patients in the present study. However, the mean serum concentrations of LDL cholesterol and triglycerides in the study subjects were higher than those outlined in the National Cholesterol Education Program (NCEP) guidelines for patients at high risk of CAD (17). The importance of hyperglycemia as a risk factor for vascular disease, particularly in the presence of hyperlipidemia, has been demonstrated outside the context of transplantation (17–19). Our results suggest a similar association for TxCAD. Specifically, in our multiple logistic regression analysis, HbA_{1c} was the only significant predictor of TxCAD, defined as ≥70% stenosis, as revealed by angiography, and it was the most powerful predictor of TxCAD, defined as MIT ≥0.3 mm, as determined by ICUS, in a model that included donor age, rejection score, ΔBMI, and serum total cholesterol.

Study limitations. One of the limitations of this or any study of TxCAD is the definition of the disease itself. One or more years after heart transplantation, most patients show ultrasound evidence of intimal thickening that is not apparent on the angiogram (11); the "gold standard" for diagnosis of this clinical entity is therefore ICUS. However, only a minority of the patients in the present study underwent ICUS; inclusion in the study was based on conventional coronary angiography. In addition, only the region from the left main coronary artery to the mid portion of the left anterior descending coronary artery was examined in the patients who underwent ICUS in our study. Furthermore, measurements of intimal thickness were taken at a limited number of sites in each patient, thus reflecting the disease process only at these sites. However, TxCAD is diffuse in nature (20,21), so that observation of the left anterior descending coronary artery likely provides a reasonable indication of the extent and severity of the disease throughout the remainder of the coronary arterial tree, corresponding to angiographic severity.

These limitations are mitigated by the significant correlation of MIT with the angiographic data in the patients of the present study. Although the presence of any angiographically detectable disease has been proposed as a preferred cutoff for diagnosis of TxCAD, our analysis showed a high degree of concordance between intimal thickening (≥0.3 mm) and the presence of mild, moderate, or severe angiographic disease. Our rationale for not using any angiographic stenosis as the cutoff is further supported by the observation that there was less concordance between intimal thickening and any angiographic disease, suggesting that intimal thickening may not have been present in angiographic lesions with ≤30% stenosis, and that these minor lesions might be due to coronary spasm.

Another limitation of our study is that it was not able to address the outcome of TxCAD. Furthermore, the patients included in the analysis constitute a selected group in that they had survived for >3.5 years after transplantation, and that individuals who developed TxCAD later than 4.7 years after transplantation were also excluded.

Classifications of coronary disease severity allow grouping of patients according to the degree of vessel wall involvement but do not take into account the fact that the disease process is not categorical. An important aspect of any discontinuous classification system is selection of cutoff values between grades. The present study relied predominantly on the classification determined by conventional angiography. Our results might therefore have been biased, because focal epicardial stenoses may have been the result of conventional atherosclerosis or even present in the donor heart at the time of transplantation; thus, such stenoses may or may not have been related to the immune phenomena thought to underlie much TxCAD. Our rationale for use of angiographic classification in this retrospective study was based on the fact that most transplant patients undergo coronary angiography and therefore could be included to allow a robust statistical analysis of risk factors. Intravascular ultrasound data were available only for a subset of patients, because this procedure is not routinely performed, even though it is more sensitive for the detection of TxCAD.

The definition of no disease as lesions with ≤30% stenosis is, strictly speaking, incorrect, given that one or more years after heart transplantation, most patients show ultrasound evidence of intimal thickening that is not apparent on the angiogram (13). The differentiation between

moderate (31% to 69% stenosis) and severe (≥70% stenosis) TxCAD is also arbitrary and does not necessarily correlate with the ischemic potential of lesions, as has been shown by the poor correlation between stress-induced myocardial ischemia and angiographic findings in heart transplant patients (22). In the field of clinical cardiology, however, the use of such nomenclature is accepted for communication of data and for decision-making. We defined severe TxCAD, as identified by angiography, as ≥70% stenosis or the presence of severe peripheral pruning. The pathophysiology of the diffuse process of pruning is uncertain. It may primarily represent an immune-mediated rejection-like process unlike conventional atherosclerosis. Indeed, TxCAD that develops early after transplantation progresses rapidly and portends a poor prognosis, consistent with an immune-mediated process (23).

Although the plasma concentration of HbA_{1c} was correlated with the severity of TxCAD identified by angiography, the correlation was relatively weak (Spearman $r = 0.24$, $p = 0.0434$), possibly as a result of the small number of patients in the study. It will thus be important to perform a multicenter study to confirm this potential association between glucose intolerance and TxCAD.

We used an average rejection score for each patient derived from ISHLT grading as a potential risk factor for TxCAD. The use of such a score may introduce an oversimplification into our analysis, because it is likely that biopsies of grade 2 or higher differ qualitatively (assuming random distribution of sampling error), and that the responses of the clinician to grade 2 or 3A biopsy differ, possibly affecting prescribed medications and, consequently, glucose tolerance.

Another important limitation of our study is that it did not address cytomegalovirus (CMV) as a potential covariable in the risk analysis. Infection with CMV is a risk factor for TxCAD, and prophylaxis with antiviral agents, such as ganciclovir, has been shown to reduce the incidence of TxCAD (24). In contrast to CMV disease that develops after transplantation, the correlation between pretransplant CMV serology and TxCAD is controversial. Given that we do not routinely measure CMV activation by culture, antigenemia tests, or polymerase chain reaction, we were unable to examine the correlation of CMV disease or infection with TxCAD in the current cohort of patients. From a pathophysiologic perspective, such an analysis might be important because of the propensity for CMV to induce chronic inflammation. Our decision not to include the CMV serostatus of donors and recipients in the present analysis was partly based on our previous studies showing a correlation between donor positivity and TxCAD in univariate but not multivariate analysis (16).

Conclusions. Our results indicate that persistent glucose intolerance, as reflected by an increased plasma level of HbA_{1c}, is significantly correlated with the occurrence of TxCAD, providing further evidence that glucose intolerance plays a role in the disease process. The discordance between HbA_{1c} and fasting glucose concentrations suggests that strategies that target postprandial hyperglycemia are warranted. Our observation that ΔBMI was also associated with the occurrence of TxCAD merits further study, and approaches aimed at preventing this post-transplant increase in BMI should be assessed. Finally, prospective studies are needed to determine which immunosuppressive drugs are least likely to induce this clinically important abnormality.

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